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RECONSTITUTION OF NATIVE COLLAGEN FIBER FROM ACID PRECURSOR GELATIN

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This invention relates to collagen. Particularly it relates to the reconstitution of native collagen fibers from selected water soluble disorganized high-molecular weight gelatins, and to the reconstituted fibers.

Native collagen which occurs mainly in animal connective tissues such as skin and tendons consists of fibers exhibiting a typical striated or banded structure with an axial periodicity of 640 A. (A.=1 Angstrom unit=1×10^{-8} cm.) when viewed in the electron microscope.

Collagen fibers consist of fibrils or bundles of single peptide chains, protofibrils, which are organized into highly oriented three-chain coils. A variety of rather mild thermal and chemical treatments denature native collagen.

A soluble collagenous protein known as procollagen or tropocollagen can be extracted from animal connective tissue by acid at low temperatures. Schmitt et al. discovered in 1942 (J. Cellular Comp. Physiol. 20:11) that it was possible to reconstitute collagen fibrils from a solution of the low temperature acid soluble tropocollagen portion of animal tissue. The tropocollagen molecules exist in solutions as the three chain coiled rods of peptide chains. Upon neutralization or salting out of such a solution, these molecules reaggregate into fibers possessing identity periods and the fine structure characteristic of native collagen.

Most native collagens, unlike tropocollagen, are not acid soluble at low temperatures. Heat must be applied to put the collagen into solution, but this treatment melts out the collagen structure and yields water-soluble gelatin. During this melting the collagen fibers are broken up into single peptide chains or protofibrils, each chain having a disorganized random-coil configuration. Heretofore the universal experience wtih gelatin has been that the degradation of collagen molecules into disorganized single chain protofibrils is irreversible, there being no recognized method for reorienting the disorganized chains back to the complete native collagen structure.

We have discovered that melting of the collagen structure may be controlled so as to produce peptide filaments consisting of disoriented networks of single peptide chains which are cross-linked together. By a certain process disorganized networks may be reorganized to form fibers exhibiting the properties of native collagen.

A primary object of this invention is to provide a novel method for reconstructing collagen fibers from solutions of cross-linked gelatins. A second object is to provide collagen fibers having increased reactivity, and which are amenable to chemical and physical manipulation to provide new and superior types of collagenous products. This invention has the important advantage of utilizing collagenous scrap and low quality leather stock as a source of high quality manipulatable collagen stock. Other objects and advantages will appear as this specification continues.

Our invention provides a novel method for utilizing collagen, collagenous waste material, hide scrap, and the like as a source of valuable native collagen fibers. These fibers may be spun into threads, molded into sheets, or fabricated into other forms. Glue or gelatin stock may be treated to obtain therefrom the molecular species used in the present process. After extraction of the molecular species that can be reconstituted, the residue of the stock

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can be further treated to extract usual gelatin or glue of commerce.

Reconstituted collagen fibers of this invention have many desirable properties not found in native collagen. Extracted fibers of the present invention because of their physical state of dispersion react with various reagents more readily and completely. They are apparently more reactive than native collagen fibers due to their smaller diameter and the ease with which various reagents penetrate their interstices. Our reoriented collagen fibers may be used to build leather and other products having desired properties making production of new leather-like products possible. Our fibers may be extruded into threads which after tanning yield high quality ligatures and textile fibers.

Although any animal tissues containing high levels of collagen are suitable as starting material for the practice of this invention, we prefer to employ mammalian corium. Skins, hides, ossein, reticular tissue and tendons are examples of suitable collagenous starting materials. It is important that the collagen used as a starting material comes from a source which has never been subjected to liming treatment.

The collagenous raw material should be subjected to a certain degree of purification. Substantially all fat, dirt, flesh, and the like should be removed from the collagen before gelatin extraction. Soaking hides in 10% sodium chloride solution followed by thorough washing to remove most of the salt is a simple and satisfactory purification method.

Typically the process of this invention includes heat and acid extraction of high molecular weight gelatin from collagen, followed by acid contacting of the cooled gelatin, removal of the acid from the gelatin to thereby cause fiber formation in the gelatin, and separation of the fibers from the gelatin.

The gelatin from which collagen fibers may be reconstructed can be described as high molecular weight cross-linked acid-precursor gelatins having a weight average molecular weight of above about 200,000. Gelatin of this description may be extracted from collagen by heating the collagen to temperatures of from about 50° C. to about 80° C., at pH's of from 6.6 to 2.2, for times ranging from about 1 to 3 hours. We prefer to extract the gelatin by heating a collagen slurry to 60° C. at pH 6.0 for about 1 hour. Preferably a mineral acid such as hydrochloric acid is used to acidify the collagen, but other suitable acids may be employed.

Although we prefer not to do so, lower temperatures as from about 30 to 60° C., may be used during gelatin extraction provided a rather concentrated solution of a hydrogen-bond competing water soluble material is added to the collagenous raw material. Examples of suitable solutions of this description include 4 to 6 molar urea and 2 molar potassium or sodium thiocyanate.

Following extraction, the high molecular weight gelatin is cooled to below about 10° C. and contacted with the water solution of an organic acid which is also at a temperature of below about 10° C. Examples of suitable organic acids are acetic, citric, glycolic and propionic. The concentration of the acid solution may suitably be within the range of about 0.05 to 0.2 M. We prefer to use acetic acid having a concentration of about 0.15 M, or 0.1 M citric acid solution.

We may use one of several methods for contacting the gelatin by the cold organic solution. Preferably we add the acid solution directly to the chilled gelatin. We may, however, first dissolve the gelatin in a warm salt solution and dialyze this gelatin solution against the cold organic acid solution. This alternate procedure ultimately yields collagen fibers which have the typical 640 A. banding of native collagen but in addition have unusually small diameters.